Effects of Sunlight and Simulated Rain on Residual Activity of *Bacillus thuringiensis* Formulations

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ABSTRACT The effect of sunlight and simulated rain on the residual insecticidal activity of *Bacillus thuringiensis* subsp. *kurstaki* formulations applied to field grown cabbage were measured. Leaf samples were collected 1, 2, 4, and 7 d after treatment and assayed against neonate cabbage looper, *Trichoplusia ni* (Hübner). Simulated rain applied by a sprinkler irrigation system washed *B. thuringiensis* from the plants, causing on average 20% reduction in insecticidal activity across all treatments. Sunlight intensity was manipulated by applying degrees of shade treatments consisting of no cover, clear plastic covers, and black plastic covers. Black plastic provided protection from sunlight degradation for 7 d, whereas both clear plastic and no cover treatments lost insecticidal activity equally beginning 2 d after application of *B. thuringiensis*. There was no interaction between simulated rain and shade treatments and thus no synergistic loss of insecticidal activity by the combination of both environmental factors. Formulations of *B. thuringiensis* differed in their ability to resist wash-off by simulated rain and degradation by sunlight. Formulations consisting of 1% wt:vol gluten or 0.5% wt:vol casein resisted wash-off better than flour/sucrose (2% wt:vol) and Dipel 2X. Resistance to sunlight degradation was greatest with the gluten formulation and progressively less for casein, flour/sucrose and Dipel 2X formulations. Half-life of insecticidal activity in response to sunlight was calculated to be 7.1, 5.7, 4.8, and 4.3 d for gluten, casein, flour/sucrose and Dipel 2X formulations, respectively.

KEY WORDS *Bacillus thuringiensis, Trichoplusia ni*, formulation, rain, sunlight

CONTROLLING INSECT PESTS with ecologically benign techniques is gaining in popularity. Inundative applications of microbial pesticides effectively control insect pests and are safe for nontarget animal species. Microbial pesticides continue to become more economical for growers as efficacy and reliability of these applications improve as a result of pathogen selection and processing. Pesticides made with the bacterium *Bacillus thuringiensis* subsp. *kurstaki* are effective for controlling a variety of lepidopteran pests (Lereclus et al. 1993). Yet, environmental conditions in the field adversely affect the insecticidal activity of *B. thuringiensis* (Frye et al. 1973, Leong et al. 1980, Dunkle and Shasha 1989, McGuire et al. 1994, McGuire and Shasha 1995). Rain physically removes spores and crystals from the target site, reducing residual insecticide activity of the *B. thuringiensis* application (Cantwell 1967, Frye et al. 1973, McGuire et al. 1994, McGuire and Shasha 1995). Light energy destroys spore viability (Griego and Spence 1978, Pinnock et al. 1971, Frye et al. 1973), degrades the toxic protein (Pozsgay et al. 1987), and reduces insecticidal activity (Raun et al. 1966, Leong et al. 1980). Both rain and sunlight render the application ineffective by reducing the residual activity of pesticide treatments. Among the studies cited thus far, only Frye et al. (1973) observed the relative effects of rain and sunlight under field conditions by recording viable spore counts from plastic garden labels treated with *B. thuringiensis*.

It has been suggested that efficacy and residual activity of entomopathogen applications could be increased by improving the formulations (Griego and Spence 1978, Pinnock et al. 1971, Raun and Jackson 1966, Cantwell and Franklin 1966). Formulations of *B. thuringiensis* made with wheat-gluten (Behle et al. 1997), a mixture of flour with sucrose (McGuire and Shasha 1990, 1995), and casein (Behle et al. 1996) have been shown to extend residual insecticide activity under laboratory conditions. These formulations retain a greater level of insecticidal activity than a commercial formulation when exposed to simulated rain and simulated sunlight in laboratory assays. The following study was conducted to evaluate residual insecticidal activity for several formulations made with *B. thuringiensis* when exposed to specific combinations of rain and sunlight under field conditions. Rain was simulated by a sprinkler irrigation system and sunlight was manipulated with the use of plastic covers.

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Materials and Methods

We measured the residual insecticidal activity of *B. thuringiensis* formulations applied to cabbage, *Brassica oleracea* L., plants grown in a field located at the United States Department of Agriculture National Center for Agricultural Utilization Research in Peoria, IL. The research area (16.7 by 16.7 m) was planted with cabbage spaced 0.6 m apart in rows 1.2 m apart. Each plot was composed of 10 consecutive plants in a row. There were 3 plots per row and 14 rows for a total of 42 plots, but not all plots were used in a given block. Two plantings, 1 spring and 1 fall, were used for this experiment.

Formulations. Four formulations of *B. thuringiensis* were applied to the cabbage plants. Dipel 2X (32,000 IU/mg, Abbott, North Chicago, IL) was mixed to approximate the recommended rate of 1.12 kg/ha (1 lb/acre) at 3.54 g/liter. Gluten, casein, and flour/sucrose formulations were made with technical powder (69,000 IU/mg) supplied by Abbott at 1.77 g/liter. Wheat-gluten (Midwest Grain Products, Atchison, KS) was dissolved in KOH (final pH = 10.5) at a concentration of 1% wt:vol before adding the technical powder (Behle et al. 1997). Flour/sucrose consisted of dry mixing equal amounts of flour 961 (Illinois Cereal Mills, Paris, IL) with sucrose. This mixture was added to water to provide a final concentration of 2% wt:vol (McGuire and Shasha 1995). Casein (American Casein, Burlington, NJ) (0.5 g) was dissolved in 100 ml KOH (0.05%, final pH = 8.5) before adding the technical powder (Behle et al. 1996).

Application of Formulations. The entire experiment was replicated 3 times (blocks). Two applications of *B. thuringiensis* formulations (6 and 20 June 1994) were evaluated on a spring planting of cabbage plants and 1 application (6 September 1994) was evaluated on a fall planting. Because formulations were applied twice to the spring planting of cabbage, a sample of leaves was assayed (see leaf assay below) before the 2nd application of formulations to ensure there was no residual insecticidal activity remaining from the 1st application. Formulations were applied with a CO_2 charged backpack sprayer at 118 ml per plot, 3.5 kg/cm^2, and 4.6 km/h through an adjustable row application kit (#23770, Spraying Systems, Wheaton, IL). The kit directed 3 nozzles (8X Cone Jet, Spraying Systems) toward the row with 1 nozzle over the row and 1 nozzle directed downward at a 45-degree angle on each side of the row. Formulations were applied during the early morning (0630–0700 hours) before wind speeds exceeded 5 km/h. Formulations were allowed to dry before the application of simulated rain.

Simulated Rain. The plot area was divided in half (split plot design) with ½ receiving simulated rain applied by a Rain bird (Rain Bird Agri-Products, Glendora, CA) sprinkler irrigation system. Plots were treated with rain on the same day as formulation application after the formulations had dried on the plants. Impact sprinkler heads were mounted on PVC pipe at 60 cm above the soil. Six heads, arranged in 2 series of 3 heads each, were run 1 series at a time at a pressure of ≈3.5 kg/cm^2. The irrigation pattern was designed for 100% overlap to provide uniform coverage after both series were run. Irrigation was measured by 6 rain gauges placed randomly among plots receiving water. Rain was simulated by the application of ≈3.2 cm of irrigation applied in ≈3 h. When natural rainfall was imminent, all uncovered plots were covered with clear plastic to prevent wash-off of the formulations and uncovered when the sky cleared. Thus, the cabbage did not receive natural rain during the course of this study.

Shade Treatments and Light Measurements. Three shade treatments were applied to the plots after simulated rain was applied. Tent-shaped frames made of PVC pipe (1.9 cm diameter) were erected over the plots and covered with either clear or black polyethylene-based plastic. The no-shade treatment was not covered with plastic and received ambient light. A full shade treatment consisted of covering the plot with a sheet of 3 mm thick black plastic cut to 120 cm wide and tented over the plots. Ends of the tents were left open and the sides of the plastic tents were clipped 5–10 cm above the soil level to allow for air circulation. Clear plastic (4 mm thick) was cut to a width of 120 cm and tented over respective plots to provide air flow conditions similar to the black plastic tents. Plastic tents remained in place for 7 d. Light energy (W/m^2) under each of the shade treatments was recorded using a Spectroradiometer model 1800 (LiCor, Lincoln, NE). Measurements were recorded 6 June 1994, at 1530 hours and between 1200 and 1300 hours on 20 September 1994. Weather conditions were characterized as clear skies and bright sun with no visible clouding. A radiometer/photometer (International Light, Newburyport, MA) was fitted with a UV detector model SEL 240 (International Light) to measure the energy (μW/cm^2) between 220 and 315 nm.

Leaf Assay. Residual insecticidal activity for each treatment combination was determined 1, 2, 4, and 7 d after application. On each collection day, 10 cabbage leaves from each plot were returned to the laboratory and assayed against neonate cabbage looper, *Trichoplusia ni* (Hübner). A 33-cm^2 circular disk was cut from each leaf and placed individually in petri dishes with 10 larvae. Dishes were wrapped with parafilm to seal the edges and placed in a dark incubator at 28°C. After 3 d, the number of live and dead larvae were counted and the percentage mortality for each treatment was obtained. A separate untreated control plot was established for each combination of cover and rain and leaf disks were collected and assayed as above to determine differences in control mortality attributable to environmental conditions. Control mortalities were analyzed separately by analysis of variance (ANOVA) to determine if differences in plant quality caused by shade or rain, or both, might affect the ability of

T. ni neonates to survive on the cabbage foliage. Because differences did occur, control mortalities from each rain*cover treatment were used to correct percentage mortality from similarly covered plants treated with formulations containing *B. thuringiensis* (Abbott 1925).

A split plot design assigned plots in half of the experimental area to either rain or no rain treatments. Then, plots within each half were assigned randomly to a shade treatment (no plastic cover, clear plastic cover, or black plastic cover) and a formulation treatment (untreated, gluten, flour/sucrose, casein, Dipel 2X) for a total of 30 combinations of treatments applied in each of 3 blocks conducted over the summer. The location of the rain treatment was alternated among blocks and the shade and formulation treatments were randomized for each block. Treatments were evaluated based on the split plot design for rain treatments, with block, light, and formulation as main effects, and each plant (leaf disk) as a subsample for a given day. ANOVA of corrected mean percentage mortality was determined for *B. thuringiensis*-treated plants only using PC-SAS version 6.04 PROC GLM (SAS Institute 1989) with rain, shade, formulation, and day as the main effects. Treatment means and standard errors were calculated for each sample date using the MEANS option. To compare differences among means of interest, the least-squares means option was used. To evaluate the effect of a one-time rain event without the effects of sunlight, Abbott-corrected results from the day 1 sample were analyzed independently from the remainder of the sample days. This information is useful when developing formulations to resist wash-off. The rate by which insecticidal activity was lost was determined by regression of mortality means over time. PROC REG was used to determine the regression slope and this slope represented the loss of insecticidal activity over time. Slopes for each of the formulations were compared based on a *t*-test and were determined to be different if $P \leq 0.05$. Each plant was considered a subsample of the plot and each application of formulations was considered a block.

Results

Weather conditions recorded by the National Weather Service at the Greater Peoria Airport, located ≈8 km SW of the research area indicated normal temperatures during June. Both the lowest temperature of the month (9°C on 10 June) and the lowest average daily temperature (15°C on 8 June) were recorded during the 1st block of this experiment. The highest temperature during the experiment was 35°C recorded on 20 June. The weather station recorded 0.5 cm of rain during the week of the 1st block, 5.6 cm during the 2nd block as a result of thunderstorms and no precipitation during the 3rd block. Actual rainfall on the plots may have varied somewhat from rainfall recorded by the weather station. However, cabbage plants were covered during the threat of rain so that none of this natural precipitation affected the study. Percentage of sky cover ranged from 4 to 98% and averaged 62, 56, and 90% for the 7 d corresponding to each of the 3 blocks. Thus, the weather during the course of this study represents a variety of seasonal conditions.

The results showed significant differences ($F = 14.09$; df = 2, 279; $P < 0.0001$) for overall larval mortalities not corrected for control mortality among the 3 blocks with block 1 (68%) averaging a greater mortality (least-squares means $P < 0.05$) than either block 2 (63%) or block 3 (62%). However, when larval mortalities were corrected for control mortalities using Abbott's formula, blocks 1, 2, and 3 averaged 69, 71, and 66% mortality, respectively ($F = 3.33$; df = 2, 217; $P = 0.0375$).

Control plots had mortalities of cabbage looper larvae that ranged from 4 to 36% and averaged 20.6% overall (Table 1). Application of simulated rain to untreated plots did not affect the observed mortality within those plots. Mortality from plants treated with no rain averaged 22.9 ± 1.3% (mean ± SD), which was not significantly ($F = 2.79$; df = 1, 55; $P = 0.1004$) different from the plants in untreated plots that received rain (17.7 ± 1.4) (Table 2). However, untreated (i.e., no *B. thuringiensis*) plants covered with black plastic caused significantly (least-squares means, $P < 0.05$) less mortality (12.6 ± 1.7) than plants covered with clear plastic (23.7 ± 1.6) or no plastic (24.6 ± 1.6) ($F = 4.17$; df = 2, 55; $P = 0.0207$). ANOVA indicated no significant interaction between cover and rain treatments ($F = 0.52$; df = 2, 55; $P = 0.5976$) for larval mortalities in the plots without *B. thuringiensis* treatment (Table 2). The above F values were based on the model error term with 55 degrees of freedom. When the F values for the effects of rain and cover were determined using respective rep*rain or block*cover interactions as the error term, then neither main effect was significant (rain, $F = 2.46$; df = 1, 2; $P = 0.2573$; cover, $F = 2.64$; df = 2, 4; $P = 0.1860$).

Because the control mortalities were significantly greater than zero (least-squares means) $P < 0.05$), and some environmental treatments significantly affected larval mortality among the plots without *B. thuringiensis* the rest of the results presented are based on values corrected for control mortality. Simulated rain reduced the insecticidal activity of *B. thuringiensis* by 20% over all the formulations and days of evaluation ($F = 113.39$; df = 1, 217; $P \leq 0.0001$) (Table 3). ANOVA gave a highly significant rain effect and rain*formulation interaction demonstrating that rain affected persistence over all treatments and that some formulations resisted wash-off better than others. The lack of significant day*formulation and rain*day*formulation interactions suggest that the rain caused a one-time wash-off and that no further change in activity occurred after the initial rain event. Therefore, to observe the effect of rain with minimal influence from light exposure, mortalities from samples collected 1 d after application were analyzed separately. Analysis

Table 1. Mean ± SD mortality of cabbage looper larvae exposed to cabbage leaves treated with formulations of *B. thuringiensis* and exposed to rain and shade treatments

Formulation	Cover	Simulated rain	*N*	Days after application of treatments			
				1 d	2 d	4 d	7 d
Casein	Black	No rain	3	94 ± 3	96 ± 2	93 ± 4	85 ± 6
Casein	Black	Rain	3	88 ± 4	93 ± 5	79 ± 11	82 ± 12
Casein	Clear	No rain	3	94 ± 2	84 ± 13	79 ± 15	42 ± 11
Casein	Clear	Rain	3	85 ± 9	87 ± 2	69 ± 14	44 ± 4
Casein	None	No rain	3	94 ± 3	92 ± 8	78 ± 17	57 ± 3
Casein	None	Rain	3	93 ± 8	89 ± 3	73 ± 18	55 ± 10
Dipel 2X	Black	No rain	3	95 ± 4	96 ± 5	81 ± 12	78 ± 8
Dipel 2X	Black	Rain	3	72 ± 10	76 ± 6	61 ± 22	55 ± 9
Dipel 2X	Clear	No rain	3	90 ± 10	86 ± 12	61 ± 0	36 ± 3
Dipel 2X	Clear	Rain	3	73 ± 17	60 ± 23	35 ± 9	19 ± 2
Dipel 2X	None	No rain	2	92 ± 1	79 ± 2	53 ± 18	24 ± 15
Dipel 2X	None	Rain	3	68 ± 22	59 ± 15	42 ± 16	41 ± 5
Flour sucrose	Black	No rain	3	98 ± 1	98 ± 1	89 ± 4	95 ± 2
Flour sucrose	Black	Rain	2	89 ± 4	88 ± 2	79 ± 1	62 ± 10
Flour sucrose	Clear	No rain	3	98 ± 2	96 ± 2	88 ± 6	47 ± 12
Flour sucrose	Clear	Rain	3	80 ± 4	55 ± 19	56 ± 15	25 ± 10
Flour sucrose	None	No rain	3	98 ± 2	97 ± 2	78 ± 2	51 ± 11
Flour sucrose	None	Rain	3	69 ± 8	59 ± 13	38 ± 11	28 ± 16
Gluten	Black	No rain	3	98 ± 1	97 ± 3	95 ± 2	82 ± 12
Gluten	Black	Rain	3	94 ± 2	93 ± 5	86 ± 6	78 ± 16
Gluten	Clear	No rain	3	93 ± 3	97 ± 2	80 ± 8	55 ± 15
Gluten	Clear	Rain	3	89 ± 4	84 ± 4	68 ± 13	49 ± 7
Gluten	None	No rain	3	92 ± 8	96 ± 4	76 ± 9	70 ± 17
Gluten	None	Rain	3	88 ± 7	86 ± 9	58 ± 7	24 ± 6
Untreated	Black	No rain	3	21 ± 16	23 ± 31	7 ± 4	10 ± 10
Untreated	Black	Rain	3	8 ± 2	6 ± 2	13 ± 5	10 ± 10
Untreated	Clear	No rain	3	30 ± 18	36 ± 27	23 ± 16	21 ± 12
Untreated	Clear	Rain	3	26 ± 21	29 ± 22	16 ± 13	4 ± 2
Untreated	None	No rain	4	34 ± 13	29 ± 13	18 ± 13	18 ± 11
Untreated	None	Rain	3	32 ± 9	18 ± 7	23 ± 29	21 ± 22

N, number of blocks.

of the day 1 sample mimicked the analysis of the combined data over all sample days in that the application of simulated rain reduced the observed larval mortality by 20% (no rain treatment = 94% mortality, rain treatment = 74% mortality). Also for the 1-d sample, ANOVA indicated a significant interaction ($F = 3.81$; df = 3, 10; $P = 0.0468$) between simulated rain and formulation suggesting differences among the formulations in resistance to wash-off. For larval mortality 1 d after treatment, the application of simulated rain did not significantly reduce the insecticidal activity from plants treated with the casein and gluten formulations (Table 4). Insecticidal activity of Dipel 2X and flour/sucrose formulations was reduced by the simulated rain treatment. These data show that the residual insecticidal activity of *B. thuringiensis* exposed to wash-off may be extended by the addition of ingredients to the formulation.

Measurements of light energy recorded by the spectroradiometer for each of the shade treatments (Fig. 1) showed that the black plastic effectively shaded the cabbage plants (energy = 0.05 W/m^2, 300–1,100 nm). Clear plastic provided a small amount of shade (energy = 518 W/m^2, 300–1,100 nm) compared with measurements of direct sunlight (energy = 570 W/m^2, 300–1,100 nm). Photometer readings (220–315 nm) showed that black plastic eliminated UV light from the plants, whereas clear plastic tents allowed 58% of the energy in this

Table 2. ANOVA for the mortalities of neonate cabbage looper fed leaves from field grown cabbage in control plots (without *B. thuringiensis*) treated with simulated rain and shading

Source	df	Sums of squares	Mean square	*F*	Pr > *F*
Model	20	9,225.2	461.3	2.56	0.0031
Error	55	9,892.3	179.9		
Corrected total	75	19,117.4			
Block	2	2,526.8	1,263.3	7.02	0.0019
Rain	1	502.4	502.4	2.79	0.1004
Block*Rain	2	408.4	204.2	1.14	0.3287
Cover	2	1,498.3	746.1	4.17	0.0207
Block*Cover	4	1,136.4	284.1	1.58	0.1927
Cover*Rain	2	186.9	93.5	0.52	0.5976
Block*Cover*Rain	4	474.8	118.7	0.66	0.6225
Day (cover)	3	1,925.9	6,420.0	3.57	0.0197

Table 3. ANOVA for mortalities of neonate cabbage looper fed leaves from cabbage treated with formulations of *B. thuringiensis*, simulated rain and shading, after correcting for control mortality (Abbott 1925)

Source	df	Sums of squares	Mean square	F	Pr > F
Model	155	205,790.4	1,327.68	8.56	0.0001
Error	124	19,229.2	155.07		
Corrected total	279	225,019.5			
Block	2	947.0	473.5	3.05	0.0508
Rain	1	23,425.6	23,425.6	151.06	0.0001
Block*Rain	2	74.1	37.1	0.24	0.7878
Form	3	16,164.7	5,388.2	34.75	0.0001
Block*Form	6	1,811.6	301.9	1.95	0.0784
Form*Rain	3	7,257.0	2,419.0	15.60	0.0001
Block*Form*Rain	6	1,096.1	182.7	1.18	0.3223
Cover	2	27,479.7	13,739.8	88.6	0.0001
Block*Cover	4	227.9	57.0	0.37	0.8315
Cover*Rain	2	1,197.5	598.8	3.86	0.0236
Block*Cover*Rain	4	772.0	193.0	1.24	0.2956
Form*Cover	6	2,078.9	346.5	2.23	0.0441
Block*Form*Cover	12	3,320.0	276.7	1.78	0.0577
Form*Cover*Rain	6	1,981.2	330.2	2.13	0.0545
Day	3	63,898.6	21,299.5	137.35	0.0001
Block*Day	6	4,384.4	730.7	4.71	0.0002
Formula*Day	9	1,359.8	151.1	0.97	0.4646
Block*Form*Day	18	4,391.9	244.0	1.57	0.0771
Cover*Day	6	12,978.0	2,163.0	13.95	0.0001
Block*Cover*Day	12	3,584.6	298.7	1.93	0.0372
Form*Cover*Day	18	2,327.7	129.3	0.83	0.6576
Rain*Day	3	700.6	233.5	1.51	0.2163
Block*Rain*Day	6	1,565.2	260.9	1.68	0.1308
Form*Rain*Day	9	1,961.9	218.0	1.41	0.1926
Cover*Rain*Day	6	2,821.0	470.2	3.03	0.0084

See text for details related to respective controls.

range of wavelengths to penetrate, based on 0.158 ± 0.032 W/m^2 for clear plastic and 0.272 ± 0.010 W/m^2 for direct sunlight.

Analysis of variance also gave a highly significant cover effect and cover*formulation interaction demonstrating that the methods used to shade the plots were effective and that formulations provided different levels of protection from sunlight degradation of *B. thuringiensis*. The significant day*cover interaction demonstrated that the covers provided different levels of protection across all formulations. Shade provided by black plastic tents extended the residual insecticidal activity of *B. thuringiensis*. After 7 d of exposure under field conditions, *B. thuringiensis* treated plants covered with black plastic provided 76 ± 15.9% mortality of cabbage looper larvae. Plants covered with clear plastic (31 ± 17.8% mortality) and plants left uncovered (30 ± 28.0% mortality) caused significantly (least-squares means, $P < 0.05$, $n = 10$, mean-squares error = 245.6) less mortality than plants covered with black plastic. When sampling days (1, 2, 4, and 7 d after treatment) were analyzed individually, the mean mortality from plants covered with clear plastic generally was not significantly different from the mean mortality from plants left uncovered. Among the 4 sampling times, only the 4-d evaluation indicated that mortality from plants left uncovered (61 ± 21.3%) was significantly (least-squares means, $P < 0.05$, $n = 10$, mean-square error = 82.8) greater than mortality from plants covered with clear plastic (51 ± 27.0%). As stated previously, within each rain treatment 1 d after application, mortalities from leaf disks were not affected by shading treatments. However, 2 d after application, plants covered with black plastic (91 ± 8.2%) caused significantly greater mortality (least-squares means, $P < 0.05$, $n = 10$, mean-square error = 112.7) than plants covered with clear plastic (73 ± 24.7%), and plants left uncovered (78 ± 22.1%).

To characterize residual insecticidal activity for each formulation, data were analyzed for significant

Table 4. Mean ± SD mortality of cabbage looper larvae when fed cabbage leaves exposed to simulated rain treatments and formulations of *B. thuringiensis* sampled 1 d after application to field grown cabbage

Formulation	No rain	Simulated rain	$P > t^a$
Casein	92.3 ± 3.3	84.1 ± 14.9	0.2781
Dipel 2X	89.8 ± 8.1	56.3 ± 28.9	0.0011
Flour/Sucrose	97.7 ± 2.5	69.5 ± 16.5	0.0089
Gluten	92.3 ± 7.9	86.0 ± 10.4	0.4060

[a] The t-test comparing simulated-rain treatment with no-rain treatment for each formulation of *B. thuringiensis*.

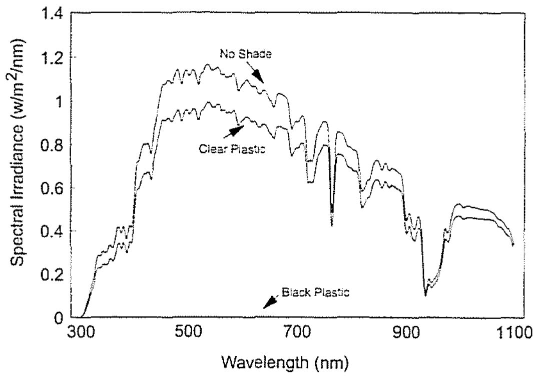

Fig. 1. Light energy recorded under shade treatments (direct sun, clear plastic, and black plastic) applied to cabbage plants after the application of *B. thuringiensis* formulations and simulated rain.

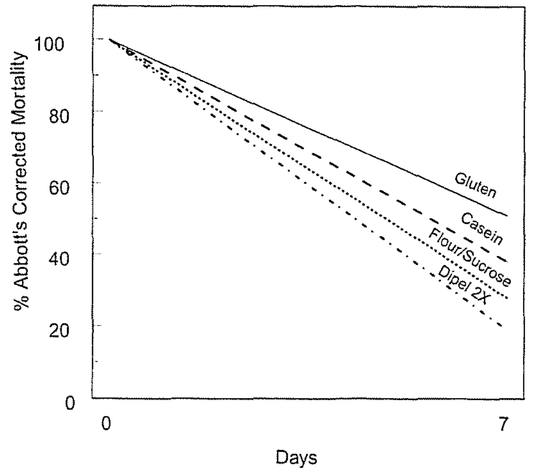

Fig. 3. Loss of insecticidal activity of formulations of *B. thuringiensis* when exposed to sunlight for 7 d (no rain treatments only).

linear regression. When covered with black plastic, the flour/sucrose formulation showed no loss of insecticidal activity based on regression analysis of the mortality over time (slope = $-0.6 \pm 0.6\%/d$, $t = 1.1$, $P = 0.3003$). Based on regression analysis, the other formulations covered with black plastic had significant ($P < 0.05$) slope estimates, but among Dipel 2X ($-3.5 \pm 1.0\%/d$), casein ($-1.7 \pm 0.7\%/d$), and gluten ($-2.8 \pm 0.8\%/d$), regression slopes were not significantly (*t*-test, $P > 0.05$) different from each other. Thus, when *B. thuringiensis* was protected from sunlight, the insecticidal activity was lost slowly, whereas loss of activity was faster when formulations were covered with clear plastic or when left uncovered (Fig. 2). These data suggest that *B. thuringiensis* would have a half-life of 14 d or longer (50% mortality ÷ 3.5%/d = 14 d) under shaded conditions.

Because the regression slopes for clear plastic and no plastic were not significantly different, these data were combined for comparing the interactions between time of exposure and formulation of *B. thuringiensis*. Results from plots that received simulated rain or were covered with black plastic were excluded for the following comparisons. Lack-of-fit tests (*F* test, $P > 0.05$) for each formulation indicated that linear regression appropriately describes the loss of insecticidal activity over the 7 d of exposure (Fig. 3). Therefore, the regression slope indicates the rate of activity lost over time of exposure in units of percentage mortality per day. Dipel 2X ($-11.5 \pm 1.4\%/d$) and flour/sucrose ($-10.3 \pm 0.9\%/d$) formulations lost activity faster than the gluten ($-7.0 \pm 1.3\%/d$) formulation. The casein formulation ($-8.8 \pm 1.2\%/d$) was intermediate. Using these slope estimates, the half-life (50% per slope) for insecticidal activity calculates to 7.1, 5.7, 4.8, and 4.3 d for gluten, casein, flour/sucrose, and Dipel 2X formulations, respectively.

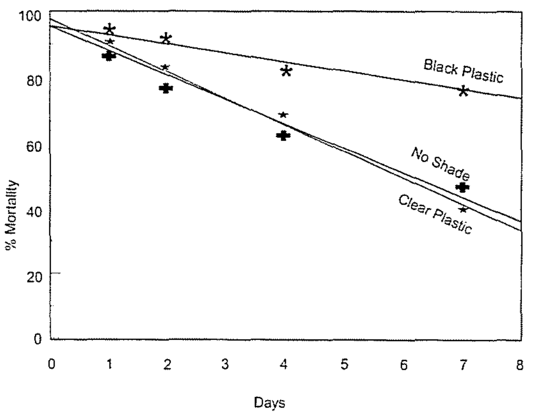

Fig. 2. Mortality of cabbage looper larvae fed cabbage leaves treated with *B. thuringiensis* and covered with clear plastic, black plastic, or with no shade (direct sunlight).

Discussion

The effects of simulated rain and natural sunlight on the insecticidal activity of *B. thuringiensis* formulations applied to field grown cabbage plants were measured. The application of simulated rain caused an immediate loss of insecticidal activity, but apparently the loss of activity was confined to the duration of the rain event. Average mortality on treated leaves from plots that received simulated rain consistently remained ≈20% less than leaves from plots that did not receive rain. This result supports the concept that rain reduces the efficacy of the pesticide application by physically removing the toxin from the target zone. There were no significant interactions between rain and duration of exposure to full or shaded sunlight. This absence of interaction between rain and light exposure indicates that these physical environmental factors act independently and are not synergistic relative to reducing residual insecticidal activity.

Sunlight rapidly degrades residual insecticidal activity of *B. thuringiensis*. Although the gluten formulation extended the residual activity of *B. thuringiensis* when exposed to sunlight, none of the formulations tested were able to compare with the residual activity expressed by plots shaded from sunlight (Table 1). This indicates that significant improvements are possible to extend the residual insecticidal activity of *B. thuringiensis* by the discovery of formulations that protect *B. thuringiensis* from degradation by sunlight.

Comparing results for plots covered with clear plastic and those with no covers shows that the clear plastic tents had little effect on the loss of insecticide activity. Therefore, it should be possible to use clear plastic as a shelter from natural rain with little to no effect relative to light degradation. This technique provides an inexpensive method to study the effects of natural rain on pesticide formulations by providing a direct comparison between rain and no rain treatments. Plots could be treated with pesticide formulations and tents erected in anticipation of a natural rain event. The materials used to construct the tents are readily available at most hardware stores, are relatively inexpensive, and are easily assembled.

Among the formulations tested in this experiment, the casein formulation provided the greatest benefits in terms of extending the residual insecticidal activity of *B. thuringiensis* if the treatment of rain and no cover is used as an indicator. Casein was also applied at the lowest rate of solids (0.5% wt:vol) of all the additives and still gave an average of 77.5% mortality over all 4 sample days. Both casein and gluten (64% mortality) were significantly better than flour/sucrose (48.5% mortality) and Dipel 2X (52.5% mortality) formulations at resisting wash-off and photodegradation.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Behle, R. W., M. R. McGuire, and B. S. Shasha. 1996. Extending the residual insecticidal activity of *Bacillus thuringiensis* with casein-based formulations. J. Econ. Entomol. 89: 1399–1405.

Behle, R. W., M. R. McGuire, R. L. Gillespie, and B. S. Shasha. 1997. Effects of alkaline gluten on the insecticidal activity of *Bacillus thuringiensis*. J. Econ. Entomol. 90: 354–360.

Cantwell, G. E. 1967. Inactivation of biological insecticides by irradiation. J. Invertebr. Pathol. 9: 138–140.

Cantwell, G. E., and B. A. Franklin. 1966. Inactivation by irradiation of spores of *Bacillus thuringiensis* var. *thuringiensis*. J. Invertebr. Pathol. 8: 256–258.

Dunkle, R. L., and B. S. Shasha. 1989. Response of starch-encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. Environ. Entomol. 18: 1035–1041.

Frye, R. D., C. G. Scholl, E. W. Scholz, and B. R. Funke. 1973. Effect of weather on a microbial insecticide. J. Invertebr. Pathol. 22: 50–54.

Griego, V. M., and K. D. Spence. 1978. Inactivation of *Bacillus thuringiensis* spores by ultraviolet and visible light. Appl. Environ. Microbiol. 35: 906–910.

Leong, K.L.H., R. J. Cano, and A. M. Kubinski. 1980. Factors affecting *Bacillus thuringiensis* total field persistence. Environ. Entomol. 9: 593–599.

Lereclus, D., A. Delecluse, and M. Lecadet. 1993. Diversity of *Bacillus thuringiensis* toxins and genes. *In* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds.], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. Wiley, New York.

McGuire, M. R., and B. S. Shasha. 1990. Sprayable self-encapsulating starch formulations for *Bacillus thuringiensis*. J. Econ. Entomol. 83: 1813–1817.

1995. Starch encapsulation of microbial pesticides, pp. 229–237. *In* F. R. Hall and J. W. Barry [eds.], Biorational pest control agents: formulation and delivery. American Chemical Society, Washington, DC.

McGuire, M. R., B. S. Shasha, L. C. Lewis, and T. C. Nelsen. 1994. Residual activity of granular starch-encapsulated *Bacillus thuringiensis*. J. Econ. Entomol. 87: 631–637.

Pinnock, D. E., R. J. Brand, and J. E. Milstead. 1971. The field persistence of *Bacillus thuringiensis* spores. J. Invertebr. Pathol. 18: 405–411.

Pozsgay, M., P. Fast, H. Kaplan, and P. R. Carey. 1987. The effect of sunlight on the protein crystals from *Bacillus thuringiensis* var. *kurstaki* HD1 and NRD12: a raman spectroscopic study. J. Invertebr. Pathol. 50: 246–253.

Raun, E. S., and R. D. Jackson. 1966. Encapsulation as a technique for formulating microbial and chemical insecticides. J. Econ. Entomol. 59: 620–622.

Raun, E. S., G. R. Sutter, and M. A. Revelo. 1966. Ecological factors affecting the pathogenicity of *Bacillus thuringiensis* var. *thuringiensis* to the European corn borer and fall armyworm. J. Invertebr. Pathol. 8: 365–375.

SAS Institute. 1989. PROC GLM user's manual, version 6 4th ed. SAS Institute, Cary, NC.

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